7.—THE SUSPENSOR AND EMBRYO OF ACTINOSTROBUS

BY ALISON M. BAIRD, B.Sc.

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The life history of *Actinostrobus pyramidalis* was described by Saxton (1913), his work being based on paraffin sections of material sent from Western Australia, but it seemed that many details of suspensor and embryo development could be more satisfactorily determined by dissection of fresh material. For this account both species of *Actinostrobus* were examined in this way.

As material of both species has been collected from plants growing in their natural habitats a few notes on the development of cones in the field are given. *A. pyramidalis* was obtained from swamps (which are dry in summer), in the neighbourhood of Perth and *A. acuminatus* from sandplains between Watheroo and Geraldton.

The period of development is long with marked resting stages associated with the summer drought. It was found that actually fourteen to fifteen months elapse between pollination and fertilisation, not three as stated by Saxton, a mistake which could be very easily made if one had not seen the plants in the field. In *A. pyramidalis* young female cones are distinguishable in December, remain very small through the summer and are found open for pollination the following July. Male cones are also visible early in December with the pollen shedding in July and August. The pollinated cones close and enlarge during the spring (September-November). There is little further development before the next winter rains. In June the sporophylls are scarcely projecting above the scales at the base of the cone and the embryosac is still minute. July to September the cones enlarge and the embryosac, still in free nuclear condition, extends to the base of the nucellus. Wall formation takes place in September, about a month before fertilisation which occurs in most plants about the second or third week in October. Embryos with long suspensors are found through November, and the embryo reaches its full size early in December by which time the cones are full size and the testa hard and brown. Cones may remain on the tree for several years before opening. In *A. acuminatus* pollen is shed about the middle of May—2 months earlier than in *A. pyramidalis*. Fertilization occurs about the end of October of the following year, so that the interval between pollination and fertilization is 17 months.

Pollination drops are formed in both species.

For this investigation fresh ovules were dissected in water under a binocular dissecting microscope. The stage of development of the embryos can be judged as soon as the prothallus is removed from the nucellus. Very young embryos show as a darker spot in the clear uniform prothallus, while after the suspensors have elongated a distinct cavity is visible, showing their position. Later stages are indicated by the collapse of the upper part of the prothallus and the presence of starch in the lower part which is also much broader than in early stages ; the whole ovule enlarging to fully twice its size at the time of fertilization.

Camera lucida drawings were made immediately while the embryos were still turgid and unplasmolysed. This method was found much more satisfactory than fixing and mounting the very delicate thin walled suspensors. Methylene blue was used occasionally to show up the nuclei in the embryo. All drawings show surface views of the embryos.

The development of suspensor and embryo was found to be essentially the same for the two species, so that except where specific differences are mentioned the following account applies to the genus.

The archegonia are lateral and deep seated in the narrow prothallus in one or two groups according to the number of pollen tubes. Two adjacent archegonia are fertilised from each tube. The proembryo as described by Saxton fills the Archegonium, the arrangement of the cells being variable and not in definite tiers.

At the earliest stage at which embryos are recognisable a group of minute cells with dense contents can be seen at the lower end of the archegonial complex. Each of these embryonic units consists of a suspensor cell and a much smaller embryonic cell. Four of these are formed from each zygote and the remaining few cells in the upper part of the proembryo disappear very early. There is no elongation of any region of the proembryo as a whole. Fig. 1 shows a proembryo as dissected from the prothallus. A certain amount of disturbance of the embryo initials is unavoidable in dissection but it has been confirmed by sections that there is no definite arrangement of the embryo initials. The small embryonic cells point in various directions. A certain amount of breakdown occurs even before any appreciable elongation of the suspensors. The embryos at the stage shown in Fig. 2 float out freely when the prothallus is dissected under water. Figs. 3 and 4 show typical embryo systems after the suspensors have begun to elongate. The large suspensor nucleus lies embedded in cytoplasm near the lower end while the upper part of the suspensor contains little or no cytoplasm. Starch is occasionally present round the nucleus but it is not a regular condition as in *Callitris*.

The suspensors are long and slender, of uniform diameter except for a slight enlargement at the upper end. The wall is thin in comparison with that in *Callitris*. The central tissue of the upper part of the prothallus has, by this time, broken down to form a cavity in which lie the coiled and folded upper portions of the suspensors. The embryonic cells are embedded in the firmer tissue below the cavity. Fig. 3 shows the lower part of the embryo as it is in the ovule. There are usually 2–5 suspensors with straight ends deeply buried in the prothallus and several shorter ones ending in the region of the suspensor coils. Occasionally a suspensor is found growing through the megaspore membrane. Suspensors with balloon-like enlargements of the ends occur in some ovules. These are usually among the shorter suspensors and always bear somewhat crushed and deformed embryos. It seems reasonably certain they never contribute the final embryo. The usual number of embryos per ovule is 8–16 according to the number of pollen tubes.

Some of the suspensors may collapse even before the first division of the embryonic cell. In the group shown in Fig. 5 all except three have collapsed. Disappearance of some of the suspensors may take place at almost any stage of development. As would be expected it is the shorter suspensors (and upper embryos) which collapse first, and it is invariably one of the embryos with long straight ends to the suspensors, usually the terminal one, which ultimately persists.

The first division of the embryonic cell probably takes place when the suspensors are about half their final length, but as the length of the suspensors differs very greatly in different cones this is difficult to determine. There is no thick inner wall round the embryonic cell as in *Biota* (1921). The first two walls are vertical, giving a tier of four cells. The next are more or less horizontal, and later ones very irregular. The walls are frequently oblique (cf. Figs. 7 and 8). Some embryos at early multicelluar stages are shown in Figs. 6–9. It can be seen that at no time is there growth from a single recognisable apical cell. Divisions take place in all of the primary quadrants.

When a considerable group of cells has been formed—the number varying within wide limits—the upper cells elongate to form embryonal tubes. Figs. 10–13 show embryos at this stage and also at slightly later stages. Figs. 11 and 12 indicate the variation in size of embryos when embryonal tubes begin to form. Both of these are terminal embryos of the same species (*A. acuminatus*) at the same scale, from different ovules. In general, upper embryos form embryonal tubes when there are far fewer cells in the embryo, but these rarely develop further. There is great variation in the number and size of cells, position of walls and relative size of suspensor and embryo. The variation within the species is too great to provide any significant difference between the species.

Following the initiation of the secondary suspensors by embryonal tubes, growth of the embryo is very rapid; repeated cell divisions giving a massive multicellular structure. An embryo system of *A. pyramidalis* with secondary suspensors in shown in Fig. 14. This also shows the enlargement of the lower end of the suspensor which is common at this stage.

In *A. acuminatus* the suspensor becomes considerably longer than it does in *A. pyramidalis*. A typical secondary suspensor of *A. acuminatus* is shown in Fig. 15, and one of *A. pyramidalis* about the same age in Fig. 16. Another difference between the species is shown at this stage in relative degrees of development of the several embryos of a group. In *A. pyramidalis* several may develop secondary suspensors (Fig. 14); in *A. acuminatus* no case has been seen where more than one embryo passed the early embryonal stage and the upper embryos often remain in the 1 to 4-celled condition.

The number of cells in the embryo continues to increase and the apex becomes smoothly rounded. Cotyledons show first as slight protuberances at the sides of the mass of small cells. Figs. 17, 18, and 19 show the developing cotyledons. It is obvious that the stem apex is conspicuous from the start and is only buried as the cotyledons approach maturity. The number of cotyledons is two, but one embryo has been found in which there were three, equally developed. In *Widdringtonia* this condition is apparently more frequent.

CONCLUSION AND DISCUSSION.

The embryology of *Actinostrobus* agrees in all essentials with that of *Callitris* (an account of which is appearing in another paper). From Saxton's work and from dissections which I have made of fairly late stages from material of *Widdringtonia cuppressoides* (kindly sent by Mr. Saxton), there is apparently a close agreement with *Widdringtonia* also.

These three genera differ, therefore, from all others so far described, in the complete absence of a prosuspensor, lending further support to the view that the Callitroideae are a natural group distinct from the rest of

the Cuppressaceae. The present investigations, however, indicate that the affinities of the Callitroideae are with the Cuppressineae rather than the Taxodineae. The embryo initials forming the bulk of the proembryo in *Actinostrobus* and *Callitris* appear to be the direct equivalent of the group of cells on the end of the prosuspensor in *Biota*, *Chaemycyparis*, etc., and the later development of the primary and secondary suspensors is very similar to that figured by Buchholz for *Chaemycyparis* (1932). The type of embryology that occurs in *Actinostrobus* could be derived from that of *Chaemycyparis* by complete suppression of the prosuspensor and earlier division of the embryo initials. That this is not an unreasonable supposition is indicated by the case of *Sciadopitys* (1931), where, although the normal prosuspensor becomes very long, in rare instances it may completely fail to elongate. The suppression of the prosuspensor and the elimination of practically all the proembryo, except the embryonic and primary suspensor cells, is probably related to the small size of the archegonia, the absence of a nutritive jacket layer and the position of the proembryo deeply sunken in the small prothallus. In *Taxodium*, *Cryptomeria* (1932a), and *Arthrotaxis* it is the primary suspensors which are absent, the embryos developing directly on the ends of the prosuspensor strands.

SUMMARY.

The suspensor and embryo are described for both species of Actinostrobus.

Cleavage polyembryony is a constant feature, four embryos being formed from each zygote.

There is no structure comparable to the prosuspensor of other genera of the Cupressaceae.

Primary suspensors become free from each other early, elongate greatly, and lie coiled in a cavity formed by breakdown of tissue in the upper part of the prothallus.

The first two divisions of the embryonic cell are vertical, the next horizontal.

A massive secondary suspensor is formed which is multicellular from the start.

The mature embryo has two cotyledons. The stem apex is conspicuous between the developing cotyledons.

The embryology of Actinostrobus agrees with that of Callitris and Widdringtonia, but is different from that of any other Conifer so far described.

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EXPLANATION OF FIGURES.

Fig. 1.—Small embryos still adhering to upper part of proembryo. X 140.

Fig. 2.—Young embryo in optical section showing details of suspensor and embryonic cells. X 140.

Fig. 3.—An embryo system of A. acuminatus at the 1-celled stage. X 25.

Fig. 4.—A pyramidalis—same. X 25.

Fig. 5.—A. acuminatus. A system with embryos at 2-celled stage, some suspensors collapsed. X 25.

Fig. 6.—4-celled embryo. X 130.

Figs. 7 and 8.—16-celled embryos. X 130.

Fig. 9.—Terminal embryos of a gp., centre one 8-celled.

Fig. 10.—Embryonal tubes just forming. A pyramidalis.

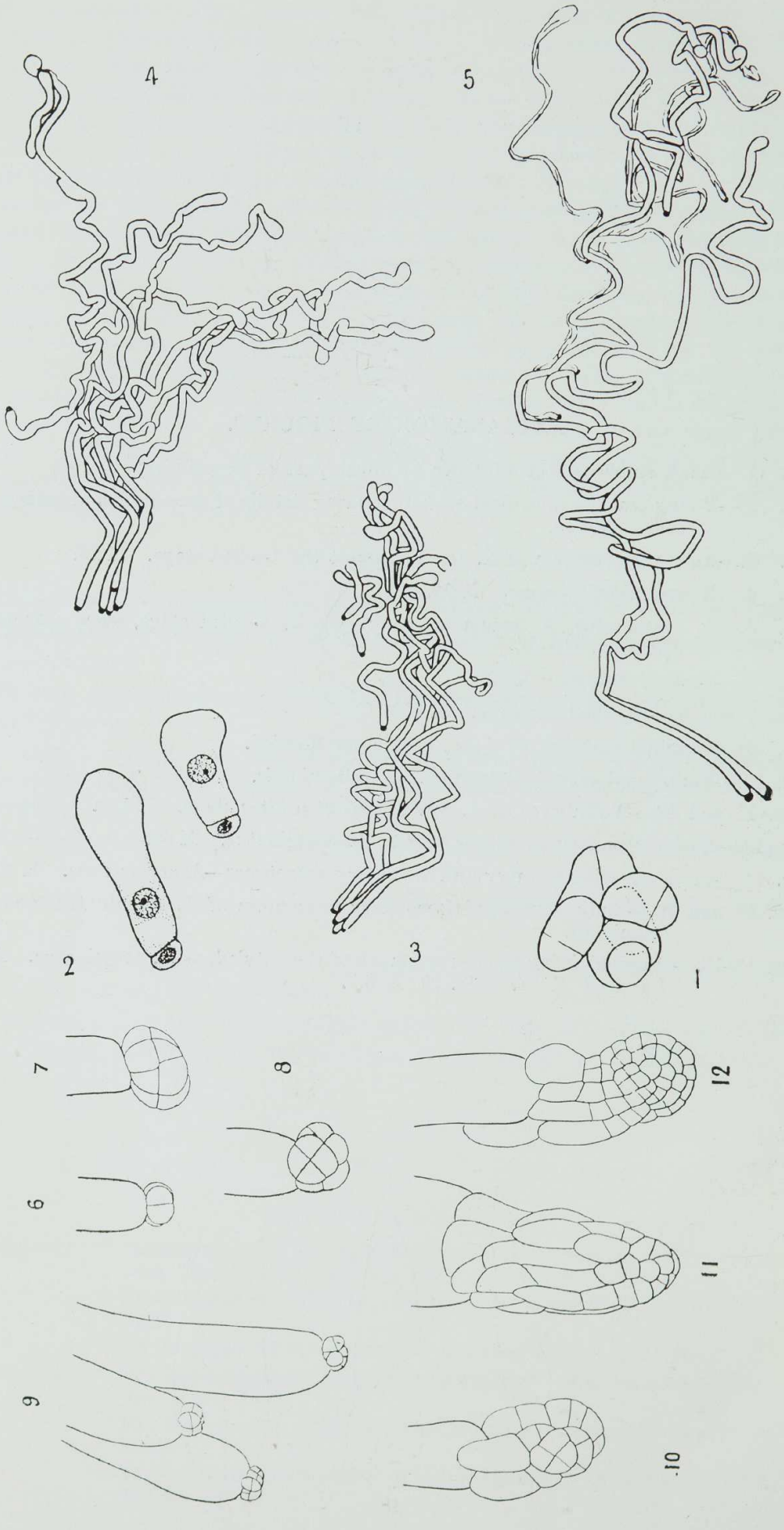
Figs. 11 and 12.—2 Embryos of A. acuminatus at a later stage. X 130.

Fig. 13.—Early secondary suspensor stage. A. acuminatus. X 65.

Fig. 14.—Group showing primary and secondary suspensors—A pryamidalis. X 30.

Figs. 15 and 16.—Fully developed secondary suspensors of A. acuminatus and A. pyramidalis.

Figs. 17–19.—Stages showing the development of the cotyledons A. acuminatus. Figs. 17 and 18 X 17. Fig. 19 X 8.5.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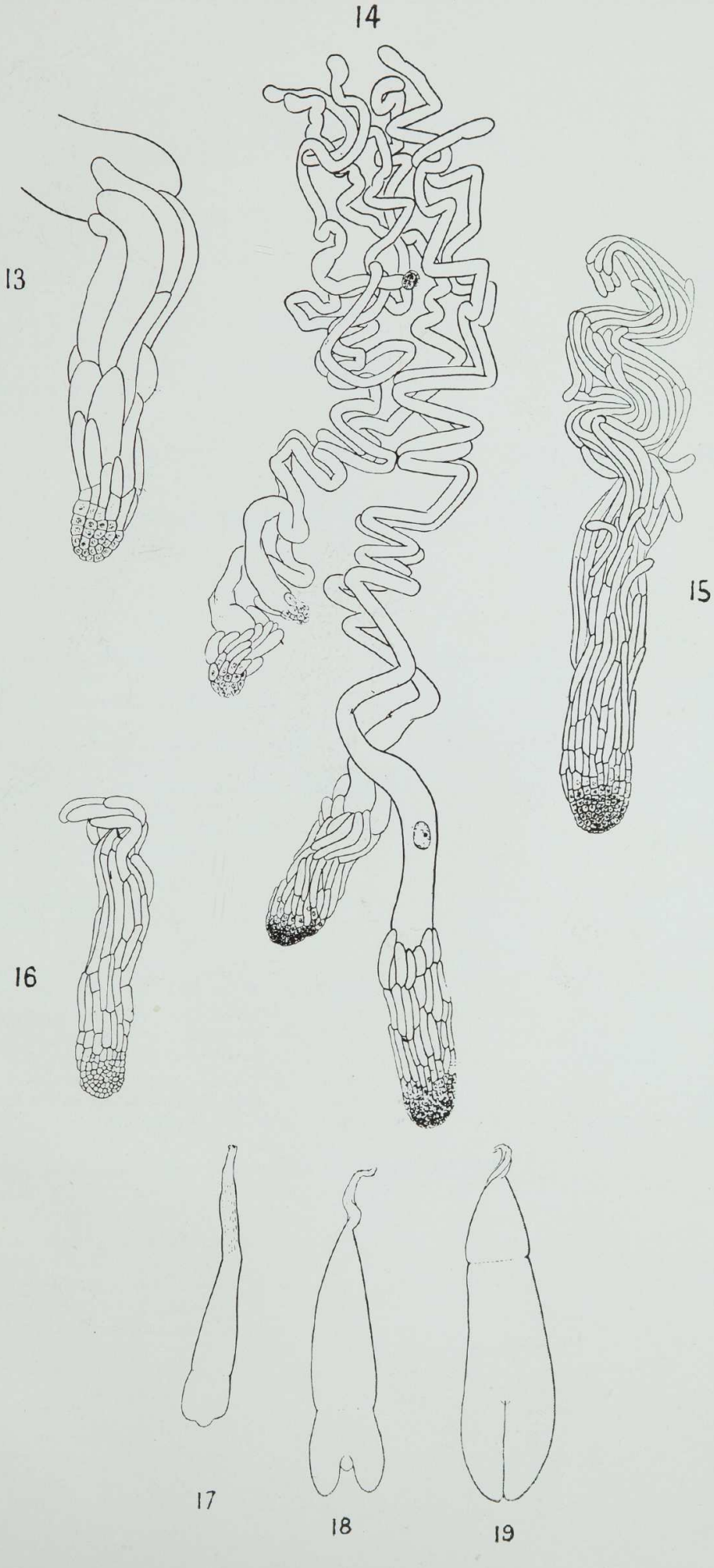
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